|  |  |  |  |
| --- | --- | --- | --- |
| DOB:  Sex: **Male**  Family #: **DMD\_0945** | MRN: **8418374**  Referring Facility:  Referring physician: **Dr. Adam Weismann**  Copies to: | Panel Coverage: **0.20%**  Avg. Read Depth: **9.21x**  Type: | Date of Collection:  Date of Receipt:  Date of Report: **(DRAFT)** |
| Test(s) Performed: **Targeted Gene Panel Sequencing** | | | |

|  |
| --- |
| **RESULT: Positive**  Findings explain patient phenotype |

**APPROACH**

Sequencing of select genes was done using Next Generation Sequencing and the data was analyzed to identify both previously classified and novel variants in targeted genes. A total of N genes with previous implications in various mendelian disorders (see Supplement for a list of genes and coverage information) were covered with minimum read depth of 30X. Note that this test cannot exclude the possibility of variants in genes not analyzed or assayed with incomplete coverage.

**VARIANTS RELEVANT TO INDICATION FOR TESTING**

One pathogenic variant in DMD was identified in this individual. No other variants of relevance to the indication were identified. Please see below for more detailed variant information.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Gene & Transcript** | **Variant** | **Allele State** | **Location** | **Disorder** | **Inheritance** | **Classification** |
| DMD NM\_004006.3 | p.Gln3585Ter | Heterozygous | Exon 75 | Duchenne muscular dystrophy | Recessive | Pathogenic |

**RECOMMENDATIONS**

The interpretation of these results should be done in the context of a patient’s medical record and family history. Please note that interpretation and classification of the variants reported here may change over time. Please see a genetic counselor for services regarding the implications of these results in the context of understanding the implications of incidental findings, family planning and the informing of family members of potentially shared genetic outcomes.

**DETAILED VARIANT INFORMATION (VARIANTS RELEVANT TO INDICATION FOR TESTING)**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Gene & Transcript** | **Variant** | **Inheritance** | | **Disorder** | **Criteria** | **Classification** |
| DMD NM\_004006.3 | p.Gln3585Ter | Recessive | | Duchenne muscular dystrophy | PM2, PVS1, PP5 | Pathogenic |
| **Location** | **Allele State** | **gnomAD All (Novel) Allele Frequency** | | | | |
| Exon 75 | Heterozygous | Novel | | | | |
| **Genomic Position** | | | **NGS Reads Supporting Change** | | | |
| g.31165436G>A | | | 4901.00% (223 of 455) | | | |
| **VARIANT INTERPRETATION:**The stop gained NM\_004006.3(DMD):c.10753C>T (p.Gln3585Ter) has been reported to ClinVar as Pathogenic with a status of (1 stars) criteria provided, single submitter (Variation ID 1383957 as of 2023-01-05). The p.Gln3585Ter variant is novel (not in any individuals) in gnomAD All. The p.Gln3585Ter variant is novel (not in any individuals) in 1kG All. This variant is predicted to cause loss of normal protein function through protein truncation. This variant is a stop gained variant which occurs in an exon of DMD upstream of where nonsense mediated decay is predicted to occur. This variant has been previously classified as pathogenic, indicating that the region is critical to protein function. There are 8 downstream pathogenic loss of function variants, with the furthest variant being 34 residues downstream of this variant. This indicates that the region is critical to protein function. The gene DMD has a low rate of benign loss of function variants as indicated by a low upper bound of the observed/expected confidence interval 0.15. The p.Gln3585Ter variant is a loss of function variant in the gene DMD, which is intolerant of Loss of Function variants, as indicated by the presence of existing pathogenic loss of function variant NP\_003997.2:p.W3\* and 1161 others. For these reasons, this variant has been classified as Pathogenic. | | | | | | |

**DETAILED VARIANT INFORMATION (INCIDENTAL FINDINGS)**

**Monogenic Disease Risk**

There were NO monogenic disease risk variants identified in this individual in genes unrelated to this individual's clinical presentation. Please see limitations for more detail.

**Carrier Status**

There were NO variants inferring a carrier status of a recessive disorder identified in this individual in genes unrelated to this individual's clinical presentation. Please see limitations for more detail.

**METHODOLOGY**

The individual’s DNA was extracted and fragmented, with fragments from the coding regions of the select gene panel targeted for amplification and sequencing. Reads from the sequence output were aligned to the human reference genome (GRCh37) using the Burrows-Wheeler Aligner (BWA). Variants to the reference were called using the Genomic Analysis Tool Kit (GATK). The variants were annotated and filtered using the Golden Helix VarSeq analysis workflow implementing the ACMG guidelines for interpretation of sequence variants. This includes comparison against the gnomAD population catalog of variants in 123,136 exomes, the 1000 Genomes Project Consortium’s publication of 2,500 genomes, the NCBI ClinVar database of clinical assertions on variant’s pathogenicity and multiple lines of computational evidence on conservation and functional impact. This test has not been cleared or approved by the U.S. Food and Drug Administration (FDA). The FDA has determined that such clearance or approval is not necessary.

**VARIANT ASSESSMENT PROCESS**

The following databases and in-silico algorithms are used to annotate and evaluate the impact of the variant in the context of human disease: 1000 genomes, gnomAD, ClinVar, OMIM, dbSNP, NCIB RefSeq Genes, ExAC Gene Constraints, VS-SIFT, VS-PolyPhen2, PhyloP, GERP++, GeneSplicer, MaxEntScan, NNSplice, PWM Splice Predictor. Analysis was reported using the to HGVS nomenclature (www.hgvs.org/mutnomen) as implemented by the VarSeq transcript annotation algorithm. The reported transcript matches that used most frequently by the clinical labs submitting to ClinVar.

**LIMITATIONS**

It should be noted that this test is limited to a limited number of genes and does not include all intronic and non-coding regions. This report only includes variants that meets a level of evidence threshold for cause or contribute to disease. Certain classes of genomic variants are also not covered using the NGS testing technology, including triplet repeat expansions, copy number alterations, translocations and gene fusions or other complex structural rearrangements. More evidence for disease association of genes and causal pathogenic variants are discovered every year, and it is recommended that genetic variants are re-interpreted with updated software and annotations periodically.

**COVERAGE STATISTICS:**

Coverage statistics were computed over 193004 targets.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Average Coverage (x) | % of Targeted Bp Covered | | | | |
| **0x** | **≥ 1X** | **≥ 20X** | **≥ 100X** | **≥ 500X** |
| **9.21x** | **99.80%** | **0.20%** | **0.20%** | **0.20%** | **0.19%** |

**CLASSIFICATION SYSTEM AND FREQUENCY THRESHOLDS**

This interpretation was preformed using the ACMG variant classification guidelines. The following recessive gene thresholds were used:

* Common Allele Frequency: 0.01
* High for Disorder Allele Frequency: 0.0015
* Extremely Rare Allele Frequency: 0.0002

The following dominant gene thresholds were used:

* Common Allele Frequency: 0.005
* High for Disorder Allele Frequency: 0.0005
* Extremely Rare Allele Frequency: 0.0001

Sub-populations were excluded from consideration if the total allele number failed to exceed 2000.

**ANNOTATION SOURCES**

This interpretation was preformed using the following annotation sources:

|  |  |  |
| --- | --- | --- |
| **Name** | **Version** | **Type** |
| *gnomAD Exomes Variant Frequencies 2.1.1, BROAD* | 2.1.1 | Frequency |
| *1kG Phase3 - Variant Frequencies 5a with Genotype Counts, GHI* | 2015-05-26 | Frequency |
| *Multiple Sequence Alignments of 100 Vertebrates, UCSC* | 2014-02-11 | Annotation |
| *CADD Scores 1.6* | 1.6 | Annotation |
| *Reference Sequence GRCh38 V2, NCBI* | 2022-10-17 | Annotation |
| *ClinVar CNVs and Large Variants 2023-01-05, NCBI* | 2023-01-05 | Annotation |
| *dbSNP 155, NCBI* | 2021-05-25 | Annotation |
| *Haploinsufficiency Predictions Version 3, DECIPHER* | v3 | Annotation |
| *DECIPHER Population CNV v9.2* | 2015-09-15 | Annotation |
| *Genomic Super Dups 2011-10-25, UCSC* | 2011-10-25 | Annotation |
| *GnomAD High Frequency CNV Regions 2019-11-25, GHI* | 2019-11-25 | Annotation |
| *gnomAD Structural Variants 2.1, BROAD* | 2019-03-06 | Annotation |
| *DGV CNVs - Gold Standard Variants 2016-05-15 v3, DGV* | 2016-05-15 v3 | Annotation |
| *1kG Phase3 CNVs and Large Variants 5b V2, GHI* | 2015-08-18v2 | Annotation |
| *Missense Badness and MPC, BROAD* | 2017-07-14 | Annotation |
| *Low Complexity Regions and Universal Mask, GHI* | 2015-03-29 | Annotation |
| *Repeating Elements by RepeatMasker, UCSC* | 2009-04-24 | Annotation |
| *Genetics Home Reference 2022-12-07, GHI* | 2022-12-07 | Annotation |
| *Clinical Genomic Database 2022-10-05, GHI* | 2022-11-01 | Annotation |
| *Reference Sequence GRCH37g1k V2, 1000Genomes* | 2022-10-17 | Annotation |
| *ClinGen Gene Dosage Sensitivity 2023-02-01, NCBI* | 2023-02-01 | Annotation |
| *ClinGen Region Dosage Sensitivity 2023-02-01, NCBI* | 2023-02-01 | Annotation |
| *ClinVar Assessments 2023-01-05, NCBI* | 2023-01-05 | Annotation |
| *ClinVar CNVs and Large Variant Assessments 2023-01-05, NCBI* | 2023-01-05 | Annotation |
| *ClinVar 2023-01-05, NCBI* | 2023-01-05 | Annotation |
| *ClinVar Transcript Counts 2023-01-05, NCBI* | 2023-01-05 | Annotation |
| *Conservation Scores Exonic, GHI* | 2020-10-02 | Annotation |
| *DECIPHER Developmental Disorders 2023-01-02, GHI* | 2023-01-02 | Annotation |
| *gnomAD - Gene Constraint 2.1.1 v2, BROAD* | 2019-03-06 v2 | Annotation |
| *Gene Identifiers and Descriptions 2022-12-19, GHI* | 2022-12-19 | Annotation |
| *Human Phenotype Ontology 2022-09-21* | 2022-09-21 | Annotation |
| *SIFT and PolyPhen2 Missense Predictions 2021-04-21, GHI* | 2021-02-10 | Annotation |
| *MONDO 2022-09-20, GHI* | 2022-09-20 | Annotation |
| *Mondo Gene Disease Association 2020-07-25, MI* | 2020-07-25 | Annotation |
| *OMIM Genes 2023-02-01, GHI* | 2023-02-01 | Annotation |
| *Orphanet Gene Associations 2022-11-28, GHI* | 2022-11-28 | Annotation |
| *Cytobands 2009-06-12, UCSC* |  | Annotation |
| *RefSeq Genes 105.20220307, NCBI* | 2022-03-12 | Annotation |
| *InterPro Regions 2018-12-12, GHI* | 2018-12-12 | Annotation |
| *ClinGen Gene Disease Validity 2022-12-07, NCBI* | 2022-12-07 | Annotation |